Contract No.: HR0011-13-C-0073

Institution: SYNTHETIC GENOMICS, INC.

Title: DIGITAL BIOLOGICAL CONVERTER

MILESTONE ACCEPTANCE REPORT (revised)

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1. Executive Summary

SGI has successfully accomplished contract Milestone A which was to automate the generation of a oligonucleotide synthesis design paradigm starting from a DNA sequence. SGI will automate the transmission of these synthetic oligonucleotide sequences to an oligonucleotide synthesizer that, in turn, activates it to start producing the oligonucleotides in order to demonstrate the technology for Milestone B.

2. Milestone Summary

SGI has successfully demonstrated Milestone A, which is to automate the generation of a synthesis paradigm starting from DNA sequence. The automated oligo design protocol works by searching for a combination of parameters that will yield a specific number of overlapping ssDNA oligos (typically ≤ 48 oligos) that are around a desired maximum length (80 bases). These parameters were chosen because 80 base oligos can be readily

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synthesized and 48 oligos can be reliably assembled into 1.5 kb fragments, in one reaction, with our methods.

Briefly, the total number of stages that the input sequence should be assembled in is calculated based on its total length. For each stage greater than 1, design parameters are then calculated to determine the total number of cuts that each fragment should be cut into so the fragments are no greater than a specific length threshold. Additionally, vector sequences and restriction sites are attached to each fragment while ensuring the restriction sites are unique to each sequence. The vector sequences serve as hooks for assembly into vector for cloning purposes, and also as primer binding domains for PCR amplification. The restriction sites are added to release these vector sequences and expose overlaps for subsequent rounds of DNA assembly. These parameters are then used to create fragments that will be used as input for the design of stage 1 oligos. For this stage, a similar set of parameters are calculated for oligo design except different parameters are combinatorially tested until the design scheme yields an even number of oligos that are all less than 80 bases in length.

To demonstrate this automated oligo design protocol, SGI submitted the 5, 386-bp phiX bacteriophage genome sequence (accession number NC_001422). The software broke the DNA sequence into four 40-bp overlapping sequences (each about 1,400 bp in length), appended 30-bp pUC19 vector sequences and NotI restriction site for vector sequence removal, and then broke each of the four sequences into about 48 overlapping 60-base oligos.

MS-A_oligo design parameters_NC_001422.txt - Notepad

File Edit Format View Help

```
Stage 2 design parameters
vector5prSeq: None
cuts: 4
overlap: 60
re: None
length: None
vector: None
vector3prSeq: None

Stage 1 design parameters
vector5prSeq: TTGCGCACGGTTATGTGGAC
vector: pSGI-BAC02
vector3prSeq: GGGCCGTCGACCAATTCTCA
overlap: 30
re: NotI
```


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A schematic depicting the results of mapping the 190 synthetic oligos designed for the PhiX174 (NC_001422) reference sequence is included at the end of this report.

For Milestone B, SGI intends to automate the transmission of these synthetic oligonucleotide sequences to an oligonucleotide synthesizer that, in turn, activates it to start producing the oligonucleotides. To achieve this milestone SGI will work closely with BioAutomation to get the oligonucleotide sequences outputted into the correct format for receipt and self-activation through software integrated within the oligonucleotide synthesizer.

3. Issues

No issues identified.

4. Technology Transitions

No technology transitions, publications, innovations, patents/patent disclosures, to report at this time.

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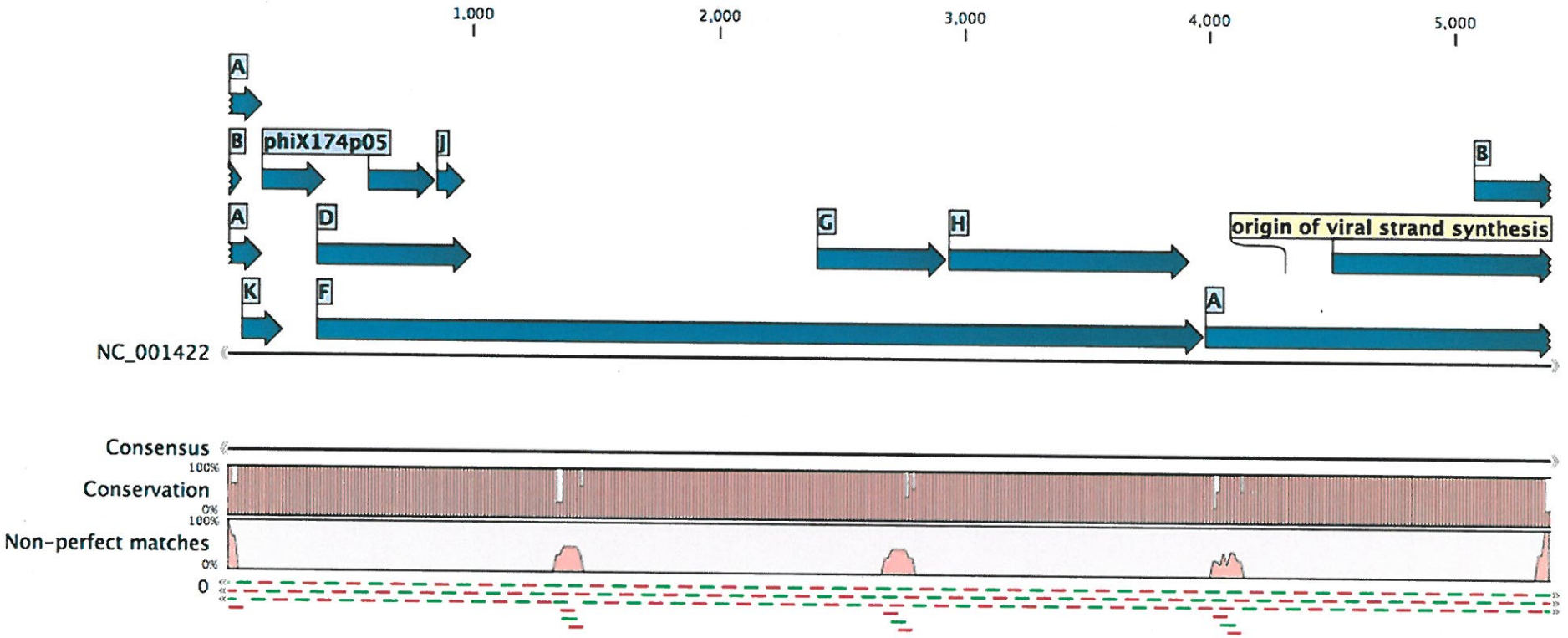
Results of mapping 190 outputted PhiX174 synthetic oligonucleotides to the NC_001422 reference sequence



- 190 oligos map to reference sequence (green line, forward; red line, reverse oligo)
- Consensus sequence exactly matches 5,386-bp reference sequence
- Non-perfect matches are only at regions where the stage 1 constructs overlap. This is expected due to the addition of vector and restriction site sequences within those oligos